

**FORENSIC TOXICOLOGY LABORATORY  
OFFICE OF CHIEF MEDICAL EXAMINER  
CITY OF NEW YORK**

**DRUGS EXTRACTED BY SPE WITH GC-NPD ANALYSIS**

**(LAMOTRIGINE, ONDANSETRON, CETIRIZINE, CLONIDINE, CLOPIDOGREL)**

**PRINCIPLE**

Solid phase extraction (SPE) is used to extract lamotrigine, ondansetron, cetirizine and clonidine which exhibit poor recovery by liquid-liquid extraction. Drug is temporarily bound to a sorbent in the solid phase column as the prepared sample is poured through these columns. The column is washed to remove interfering compounds, followed by elution of the drug. The eluent is evaporated and then reconstituted. The resulting solution is analyzed by GC-NPD. Quantitative analysis is performed using a four-point calibration curve and internal standard.

**SAFETY**

The handling of all biological specimens and reagents is performed within the guidelines which are detailed in the Safety and Health manual.

**SPECIMEN PREPARATION**

The procedure is routinely applied to the following biological specimens and their aliquots unless otherwise specified:

Blood	2.0 mL of undiluted specimen
Urine	2.0 mL of undiluted specimen
Brain	2.0 mL of a 1:3 homogenate
Gastric Contents	2.0 mL of a 1:10 dilution
Liver	2.0 mL of a 1:5 homogenate
Vitreous Humor	2.0 mL of the undiluted specimen

## DILUTION OF SPECIMENS

Specimens are diluted as follows:

Brain 1:3	5.0 g of brain homogenized with 10 mL of deionized water.
Liver 1:5	5.0 g of liver homogenized with 20 mL of deionized water.
Gastric 1:10	2.0 mL of liquid <i>q.s.</i> to 20 mL of deionized water, or 2.0 g of a solid specimen homogenized with 18 mL of deionized water.

**Note** Do not use homogenates older than two weeks unless low sample size requires it. Discuss with supervisor and note in case record.

**Note:** In preparing gastric homogenates, the entire submitted amount of gastric contents must be homogenized prior to sampling.

## REAGENTS AND MATERIALS

All chemicals should be analytical reagent grade or better.

### Deionized water (DI H<sub>2</sub>O)

#### Negative matrices

Certified negative matrices (blood, brain, and liver from calf or sheep) are obtained from outside sources and validated in-house. They are stored frozen (-10 ° C or lower).

#### 100 mM phosphate buffer (pH 6.0)

Dissolve 3.4g Na<sub>2</sub>HPO<sub>4</sub> and 24.28g NaH<sub>2</sub>PO<sub>4</sub> H<sub>2</sub>O in 1600mL DI H<sub>2</sub>O.

Dilute to 2000mL using DI H<sub>2</sub>O. Mix. Adjust pH to 6.0 ± 0.1 with 100mM monobasic sodium phosphate (lowers pH) or 100mM dibasic sodium phosphate (raises pH).

Storage: room temperature in glass.

Stability: 6 months. Inspect each day before use for contamination.

#### pH 9.0 buffer

Dissolve 20g KHCO<sub>3</sub> in 800mL DI H<sub>2</sub>O; add 10g K<sub>2</sub>CO<sub>3</sub>. Dilute to 1L using DI H<sub>2</sub>O. Mix. Adjust pH to 9.0 ± 0.1 with KHCO<sub>3</sub> to lower the pH, or K<sub>2</sub>CO<sub>3</sub> to raise the pH.

Storage: room temperature in glass.

Stability: 6 months. Inspect for contamination each day of use.

**Acetic Acid**, Glacial, HPLC grade. Fisher Scientific or equivalent.

#### **Acetic Acid, 1.0 M**

To 400ml deionized H<sub>2</sub>O add 28.6 ml glacial acetic acid. Dilute to 500 ml with deionized H<sub>2</sub>O.

Store at room temperature in glass.

Stability: 6 months. Inspect for contamination each day of use.

#### **Acetic Acid, 100 mM**

Dilute 40 mL of 1.0 M acetic acid to 400 mL with deionized H<sub>2</sub>O.

Store at room temperature in glass.

Stability: 6 months. Inspect for contamination each day of use.

**Methanol (MeOH)** - Fisher Scientific - ACS Certified

**Ethyl Acetate**, Fisher Scientific Optima or equivalent.

**Ammonium Hydroxide, NH<sub>4</sub>OH** (Fisher Scientific)

**Eluting solvent: Ethyl acetate/NH<sub>4</sub>OH (98/2).** Prepare fresh daily for use.

***Note:** Ammonium hydroxide will break down to ammonia and water and the ammonia will evaporate if the container is not kept closed. This will cause a pH decrease, making the reagent unsuitable for solid phase extraction. Use small lots of working solution (3 mL bottles). Always keep ammonium hydroxide refrigerated.*

#### **Preparation of calibrators and controls**

Following stock solutions are prepared to yield a final concentration of 100 mg/L:

<u>Drug (Manufacturer)</u>	<u>Amount (free base)</u>	<u>Volume of solvent</u>	<u>Solvent</u>
Lamotrigine (GLAXO)	0.0026g	25mL	MeOH
Ondansetron Dihydrochloride (Sigma-Aldrich)	0.0062g	50mL	MeOH
Cetirizine (Sigma-Aldrich)	0.0059g	50mL	MeOH
Clonidine (Cerillient)	1mL (1.0mg/mL) ampoule	10mL	MeOH
Clopidogrel (Sigma)	0.0033g	25ml	MEOH

All solutions are prepared in the appropriate volumetric flask.

**Polycrom Clin II Solid Phase Extraction Columns, CEREX**

**System 48 Processor** connected to nitrogen source.

## Waste Rack, SPE Rack, Collection Tube Rack.

**Concentrator (Turbovap or SPEware)** connected to a nitrogen source.

## Toluene/Heptane/Isoamyl Alcohol, 39:10:1(THI)

Add 780 mL of toluene, 200 mL of heptane and 20 mL of isoamyl alcohol to a 1000 mL graduated cylinder. Briefly swirl by hand, then transfer to a 1 L repipeter.

## EXTRACTION PROCEDURE

Calibrators and controls are prepared by adding validated stock solutions to 2.0 mL of a certified negative matrix in appropriately labeled 16 x 125 mm tubes. A four-point calibrator curve is prepared consisting of 0.05 mg/L, 0.2 mg/L, 1.0 mg/L, and 3.0 mg/L. Controls are also prepared at 0.025 mg/L, 0.5 mg/L, and 1.0 mg/L.

- 1. Calibrators:** Using the 100 mg/L validated calibrator stock solution add the following amounts to the appropriately labeled test tube
  - Add 60µL to the 3.0 mg/L cal test tube
  - Add 20µL to the 1.0 mg/L cal test tube
  - Add 4µL to the 0.2 mg/L cal test tube
  - Add 1µL to the 0.05 mg/L cal test tube
- 2. Controls:** Using the 100mg/L validated control stock solution add the following amounts to the appropriately labeled test tube
  - Add 20µL to the 1.0 mg/L qc test tube
  - Add 10µL to the 0.5 mg/L qc test tube
  - Add 0.5µL to the 0.025 mg/L qc test tube
3. After all calibrators and controls have been prepared, aliquot the case specimens. Make certain that the toxicology number on the specimen bottle exactly matches the number on the tube. Homogenize specimens if needed. Mix each room temperature specimen container by gentle inversion a couple of times and pipet 2.0 mL or 1.0 mL (dependant on specimen matrix) of each specimen into the appropriately labeled 16 x 125 mm culture test tube. After sampling all the cases, return original specimen containers to storage.
4. Add 40 µL of methapyrilene internal standard (50mg/L) to all tubes (blank, calibrators, controls and cases) then mix by Vortex.
5. Add 4.0mL 100mM phosphate buffer pH 6.0 to the tubes containing blood. Add 2.0mL 100mM phosphate buffer pH 6.0 to the tubes containing urine. Mix by Vortex for 30 seconds then sonicate for 15 minutes. Centrifuge samples for 15 minutes at  $\approx$  3000rpm.
6. Decant the supernatant into the appropriately labeled Polychrom Clin II column and apply nitrogen at a pressure of 2-4 psi.

7. Prepare the eluting solvent by pouring 98 mL of ethyl acetate into a glass flask with stopper; add 2 mL of refrigerated  $\text{NH}_4\text{OH}$ . Place flask on stir plate and allow eluting solvent to mix while completing the remaining steps of the extraction.

**Note:** Prepare fresh eluting solvent each day of use.

8. Wash column (all wash steps are pressurized at 2-4 psi).  
Pour 2 mL pH 9.0 buffer onto column (for blood samples); 1 mL for all other matrices.  
Pour 4 mL 100mM acetic acid onto column (for blood samples); 2 mL for all other matrices.  
Pour 2 mL  $\text{CH}_3\text{OH}$  onto column (for blood samples); 1 mL for all other matrices.  
Pour 4 mL ethyl acetate onto column (for blood samples); 2 mL for all other matrices.  
Dry column for 2 minutes at  $\approx 25$  psi (Max Flow)
9. Elute Drugs  
Place labeled 10 mL conical centrifuge under each column to collect eluate. Elute with 4 mL eluting solvent (ethyl acetate/ $\text{NH}_4\text{OH}$ , 98/2) for blood, and 2.0 mL of eluting solvent for all other matrices; collect eluent at 1 to 2 mL/minute. Apply pressure as necessary to achieve the desired flow rate.
10. Dry under nitrogen at  $25^\circ\text{C}$  to absolute dryness using a concentrator.
11. Reconstitute with 100  $\mu\text{L}$  of THI. Mix by Vortex and transfer to the glass insert in the appropriately labeled autosampler vial.
12. Run on GC-NPD by using the GC quantitation method; follow the "Basic Drugs Quantitation by Gas Chromatography" SOP for method and instrument set-up.
13. Process, review and report sequence using the same criteria specified in the "Basic Drugs Quantitation by Gas Chromatography" SOP.

## INSTRUMENTATION

The instrumentations used for analysis is the Agilent 6890 gas chromatograph equipped with a 7683 Series autosampler and a Agilent 6890 Series nitrogen-phosphorous detector. A computer with HP Chemstation software is used to gather the data and Agilent Data Analysis software is used to process the data.

## COLUMNS

The primary column used in the GC section is a 10.0 m x 0.53 mm x 2  $\mu\text{m}$  HP-17 (50% phenyl methylsiloxane) megabore capillary column. This column is used for most routine work, both qualitative and quantitative.

The secondary column used in the GC section are a 10.0 m x 0.53 mm x 2.65  $\mu\text{m}$  HP-5 (5 % phenyl methylsiloxane) megabore capillary column.

The third column is the RTX-50(100% methylphenyl polysiloxane). 15.0 m x 0.25mm x 0.25 um. This column is used to resolve peaks when needed.

## INSTRUMENT SETUP

### 6890 Series II Gas Chromatogram

1. Ensure that the selected GC is operational and not in need of repair. If maintenance is required, consult the appropriate manual and notify the supervisor.
2. Make sure GC power on.
3. Record status of instrument on instrument status log sheet.
4. The Chemstation method file for quantitation is usually named GCxNQNT.M, where the x refers to the instrument number. There should be a method file with the appropriate name in each method subdirectory. Check that the appropriate method file exists, and that the following parameters are correct: Use the appropriate method for the analysis, whatever the method name. Consult supervisor if correct method is unclear.
5. On Chemstation verify that all other GC parameters are set correctly; go to Method, Edit Entire Method. Wait for "Ready".

#### Method Section To Run:

- Save Copy of Method With Data
- Instrument Control Pre-Run Cmd/Macro =
- Data Analysis Pre-Run Cmd/Macro =
- Data Acquisition
- Data Analysis
- Instrument Control Post-Run Cmd/Macro =
- Data Analysis Post-Run Cmd/Macro =

#### Inlet and Injection Parameters

Sample Inlet: GC  
Injection Source: GC ALS

#### GC Injector (7683 Series Injector)

*Select Single or Dual*

*Injector Parameters (Front or Back or Both)*

Sample Washes	1
Sample Pumps	4
Injection Volume	5.0 microliters
Syringe Size	10.0 microliters

PreInj Solvent A Washes	0
PreInj Solvent B Washes	0
PostInj Solvent A Washes	5
PostInj Solvent A Washes	5
Viscosity Delay	0 seconds
Plunger Speed	Fast
PreInjection Dwell	0 minutes
PostInjection Dwell	0 minutes

### Inlet Parameters

<i>Front Inlet</i>	<i>(Split/Splitless)</i>	<i>Back Inlet</i>	<i>(Split/Splitless)</i>
Mode	Pulsed Splitless	Mode	Pulsed Splitless
Initial Temp	275 °C (on)	Initial Temp	275 °C (on)
Pressure	5.20 psi (on)	Pressure	5.20 psi (on)
Pulse pressure	10.0 psi	Pulse pressure	10.0 psi
Pulse time	0.60 min	Pulse time	0.60 min
Purge flow	30.0 mL/min	Purge flow	30.0 mL/min
Purge time	2.00 min	Purge time	2.00 min
Total Flow	48.8 mL/min	Total Flow	48.8 mL/min
Gas Saver	off	Gas Saver	off
Gas type	Helium	Gas type	Helium

### Column Parameters

*HP-17 COLUMN (Column 1 & Column 2)*

*(Select Front, Back, or Both)*

<b>Capillary column</b>			
Model number	19095L-121		
HP-50+ 50% Phenyl Methyl Siloxane			
Max Temp	310 °C		
Nominal length	10.0m		
Nominal diameter	530.00 um		
Nominal film thickness	2.00um		
Mode	Ramped pressure		
Initial Pressure	5.20 psi		
Initial Time	12.00 min		
	<b># Rate</b>	<b>Final Pressure</b>	<b>Final time</b>
1	3.20	8.20	0.00
2	0.0(off)	0.0 (off)	

Post pressure	5.20 psi	
Nominal init flow	15.4 ml/min	
Average velocity	131 cm/sec	
Inlet	Front, Back, or Both	
Outlet (detector)	Front, Back, or Both	
Outlet pressure	ambient	

### Oven Parameters (6890)

Initial temp: 120 °C  
Initial time: 2.00 min

Maximum temp: 280 °C  
Equilibration time: 1.00 min

<b>Ramps:</b>			
#	Rate (C/min.)	Final Temp. (°C)	Final Time (min.)
1	15.0	280	18.00
2	0.0(off)		
Post temp.	120 °C		
Post time	1.00 min		
Run time	30.67		

### Detector Parameters

<i>Front detector (NPD)</i>		<i>Rear detector(NPD)</i>	
Temperature	325 °C mL/min(on)	Temperature	325 °C (on)
Hydrogen flow	3.0 mL/min (on)	Hydrogen flow	3.0 mL/min (on)
Air flow	60.0 mL/min (on)	Air flow	60.0 mL/min (on)
Mode	Constant column+makeup flow	Mode	Constant column+makeup flow
Combined flow	30.0 mL/min	Combined flow	30.0 mL/min
Makeup flow	On	Makeup flow	On
Makeup gas type	Helium	Makeup gas type	Helium
Adjust offset	30.00	Adjust offset	30.00
Electrometer	On	Electrometer	On
Bead	On	Bead	On
Equilibration time	0.00	Equilibration time	0.00

### Signals

(Select Front, Rear, or Both)

<b>Signal 1</b>		<b>Signal 2</b>	
Data rate	20 Hz	Data rate	20 Hz



Type	Front detector	Type	Rear detector
Save Data	On	Save Data	On
Start Time	1.0 min	Start Time	1.0 min
Stop	30 min		30 min

After reviewing instrument parameters select a “GC Real Time Plot” for viewing on the instrument top screen while the sequence is running.

Make sure that under “Select Reports” all UNCHECKED.

Save the Method.

## SEQUENCE

Prepare a sequence using the following steps.

1. Make sure the desired instrument session is up and running. Each GC has an instrument session, such as GC1, GC2, etc.
2. From the top menu select **Sequence** → **Load Sequence** → **Default.S**. Select **Sequence** again, then **Edit**. The **Sample Log Table** will open.
3. In the upper left hand corner on the **Sample Log Table**, select **Data Path**. Click on **Browse** to create a new folder under the **Data** folder of the instrument to be used.
4. In the upper right hand corner on the **Sample Log Table**, by clicking on **Browse** select the instrument **Method** to be used for analysis.
5. Type in all information under each column (i.e. Type, Vial, Sample, Method/Keyword, Datafile, Comments/Keywordstring).
6. If the Sample is a calibrator, then under **Comments/Keywordstring** type-in the MIX or Filter it is for the batch; i.e. for all levels of CAL 1 type-in MIX [A], for levels of CAL 2 type-in MIX [B] etc. *This step will be helpful during processing the data.*
7. Repeat steps 5 and 6 until all calibrators, controls, and case samples have been entered.
8. The **Datafile** name should be read GCXMMDDx001 ; where “X” is the instrument’s number, “MM” is the month, “DD” is the day, and “x” is the prefix of the sequence, such as “a”, “b” etc.
9. Once all the information is typed, select **OK** to close the **Sample Log Table**. Save the sequence by selecting **Sequence** followed by **Save Sequence As**. . . . Name the sequence as follows: GCXMMDDYY ; where “YY” defines the year.
10. Select **Sequence** then **Simulate Sequence**. In the window that opens, select **Full Method**, **Inject Anyway**, and **Overwrite Existing DataFiles**. For **Sequence Comment** enter the instrument # and detector used, the date of injection, the analyst initials, and the type of batch being injected. Select **Run Sequence**, verify all keywords by clicking “OK”.

11. Print sequence by selecting **Sequence** followed by **Print**. .then select **Brief**. Add a "Chain of Custody" and a "Vials Loaded" label to the hard copy of the sequence.
12. Empty solvent wash bottles on the instrument and replace it with fresh methanol before starting the sequence.
13. Use the printed sequence list to load the vials in the correct position on the autosampler tray. Edit the sequence as necessary to correct any errors; save the sequence. Reprint sequence if necessary.
14. Document the "Chain of Custody" and the "Vials Loaded" labels.
15. Document the "Instrument Status Logsheet" that hangs on the instrument's oven door.
16. Start sequence by selecting **Sequence → Run Sequence**.
17. When the batch is complete, remove all vials using the sequence list. Once again document the "Vials Loaded" label.

**Note:** Occasionally, it will be desirable to run several subsequences in one batch. Use the instructions below to accomplish this.

#### **Setting-Up a Subsequence**

On the Sample Log Table:

1. Select "**Keyword**" for "Type"
2. Select "**DataPath**" for "Method/Keyword"
3. Under "Comment/KeywordString" type in the *new data path* for your subsequence ie: **C:\MSDCHEM\2\DATA\GCXMMDDYYx** where "X" is the instrument's number and "x" is the letter designated to the subsequence (**it must be different than that of the original sequence**).
4. Create a new folder for subsequence.
5. The suffix of the data files must be different from that of the original; ie: GCXMMDDx001; **the subsequence data files must start with 1 again**.
6. After typing in the entire sequence, save sequence accordingly and method your using.
7. Go to Sequence → Simulate Sequence → Run Sequence.
8. A dialog box will pop-up: DataPath **C:\MSDCHEM\2\DATA\GCXMMDDYYx** does not exist. Edit Sample Log Table? → Click **No** if the sequence was set up correctly.
9. A second dialog box will pop-up: Create **C:\MSDCHEM\2\DATA\GCXMMDDYYx?** → Click **Yes**.

10. A third dialog box will pop-up: Sequence Verification Done! View it? → Click **Yes** or **No**.
11. Save and print sequence.
12. Start sequence.

## DATA ANALYSIS

### Retrieving Method from Data Acquiring Instrument

1. Save the method to the batch from the acquiring instrument Chemstation.
2. Save method to batch by clicking ECM on the tool bar, then select Save Method to ECM. Locate the batch, select ok to save a copy of the method into the batch to be processed.
3. A copy of the method has now been saved to ECM and will accompany the batch when retrieved from ECM on any Data Analysis Processing Station.
4. Attach and document "ECM label".

### PROCESSING USING ENHANCED DATA ANALYSIS

On any processing station:

1. Click on the Processing Data Analysis icon on the desktop and Log-in by using: analyst's ocme login for the username and their password.
2. From the tool bar menu select **ECM → Retrieve Entire Sequence from ECM**. Browse to locate and select the batch. Each data file will be retrieved along with the method to be used for processing.
3. Once the retrieval is complete the batch folder is located in the C: \msdchem\ECM\retrieve folder.
4. Select the method under the batch by right clicking on it and **Load** the method.
5. Select Calibrate → Clear; clear all responses and all calibrator levels.
6. Select **Calibrate** from the tool bar, then **Update, Global Update, Set Curve Fit Type, Linear Regression Force (0, 0)**, select **OK**. If a different type of curve fit is used in place of Linear regression force (0,0), this must be annotated on the front page of the master copy of calibrators and controls that is appended to each case chromatogram. Make sure retention window setting is set to +/- 2%.
7. Set up processing Filters (Filter A = Cal 1, Filter B = Cal 2, Filter C = Cal 3, etc.
  - a. Select **Calibrate** again, **Edit Compounds**; select **Name**, type in compound name in the space provided, click **Find Compound**.



- e. Click on **Do Update** then select **OK**.
18. Load the next data file, Cal 1.0 mg/L. Type **QT 1** then click on **Execute**.
  19. Select the **View**, select **Q-Edit Quant Result**. *Easy ID is not needed for this step, as retention times have already been set for this calibrator.* Double check each of the compound's retention time and that each compound has a response. All auto-integrated compounds should have an "x" next to their name. Click **Exit** once when finished.
  20. Assign the calibrator level.
  21. Repeat steps 15-21 for the remaining levels (0.2 and 0.05mg/L) for the first calibrator.
  22. Requant the first calibrator:
    - a. Go to **Tools, DOLIST**.
    - b. Remove any options that are on the right hand side of the box.
    - c. On the left hand menu select "**Summary Quant No Report**" which is **QT 2**, with arrows move this command to the empty space on the right then click **OK**.
    - d. Select the calibrators just processed and move them to the empty space on the right then click **Process**. Each file will be "requanted".
    - e. Review all files through **Q-Edit Quant Result** to verify quant results.
  23. Process the corresponding QC's for the calibrator that was just reviewed, with the same filter on:
    - a. Go to **Tools, DOLIST**.
    - b. Remove any options that are on the right hand side of the box.
    - c. On the left hand menu select "**Quant No Report**" which is **QT 1**, with arrows move this command to the empty space on the right then click **OK**.
    - d. Select the QC's to be processed and move them to the empty space on the right then click **Process**. Each file will be processed.
  24. Review each QC by selecting **View**, select **Q-Edit Quant Result**.
  25. With the filter still on, print the Cal's and QC's for that filter:
    - a. Go to **Tools, DOLIST**.
    - b. Remove any options that are on the right hand side of the box.
    - c. On the left hand menu select "**Summary Quant w/o Calculations**" which is **QT 0, 1, "s"**, with arrows move this command to the empty space on the right then click **OK**.
    - d. Only select all Cal levels and QC's just reviewed and quanted and move them to the empty space on the right then click **Process**. Each file will print.
  26. Load the 3.0 mg/L of the next calibrator to be processed and follow steps 10-26.

27. Once all calibrators and QC's are printed, save the method to ECM, then **set the filter # to 0, which gives a list of all the compounds for the entire method, then** process all blanks and cases:
- Go to **Tools, DOLIST**.
  - Remove any options that are on the right hand side of the box.
  - On the left hand menu select "**Quant No Report**" which is **QT 1**, with arrows move this command to the empty space on the right then click **OK**.
  - Select the QC's to be processed and move them to the empty space on the right then click **Process**. Each file will be processed.
28. Review each blank and case through **Q-Edit Quant Result**:
- Deselect any compounds that are not needed in each blank or case by double clicking on it and clicking on **QDel**.
  - Check the responses and retention times of the needed compounds.
  - If a compound is needed but not integrated, double click on the compound and integrate.
  - Click **Exit** once when finished.
29. If a compound is too low to be integrated or no peak is present include an overlay:
- Go to **Tools, Overlay Chromatogram**.
  - Select the calibrator or standard to overlay on the case; multiple overlays can also be set.
  - Select **Process**.
  - Go to **File, Print** then select **Chromatogram**
30. Once all blanks and cases are processed, print all blanks and cases.
31. Print an R<sup>2</sup> report by:
- Go to **Calibrate, List** then select **Calibration Report** and **OK**.
  - Compounds that were not used in the batch can be deleted by highlighting them and simply pressing delete on the keyboard. *Caution should be used, do not remove compounds needed.*
  - Right click on the list and select **Print**, choose the correct printer.
32. Once everything is printed, save the all the data files from the batch back to ECM:
- Select **ECM** from the top tool bar, select **Save Multiple Data Files to ECM**.
  - Select all data files on the left and move them to the right empty space, the click **Process**.
  - Allow each data file to be successfully copied to ECM before exiting Data Analysis.

33. After uploading the files to ECM, delete the sequence from the C: Drive of the processing station:
- a. Right click on the **Start** on the lower left hand corner and select **Explore**
  - b. Open the **MSDCHEM** folder on the **C: Drive**, then select **1 → ECM → Retrieve**
  - c. Locate the batch processed, right click on it and delete it.
  - d. Document "ECM label" on sequence list.

## REINJECTION CRITERIA

Occasionally, samples may need to be reinjected for a variety of reasons. The criteria for reinjection, other than poor chromatography or requests made by the appropriate supervisor, are listed below.

1. Individual blood samples may be re-injected once if internal standard (IS) area fall below that of the counts for the Cal's and QC's in the batch..
2. Analytes in a calibrator or control are not present in the chromatogram.
3. Additional peaks present in the chromatogram of a calibrator or control. See TROUBLESHOOTING OF AGILENT GAS CHROMATOGRAPHS section of the SOP manual.
4. QC failure: reinject the blank, the calibrator of interest and the associated QC.
5. Examine peak shape for signs of overload. If peaks are still overloaded after reinjection, re-extract samples with overloaded peaks using appropriate dilutions. If a calibrator peak is still overloaded after re-injection, the batch must be re-extracted.

## CARRYOVER

Two types of carryover are possible with the methods employed by GC. One is the "carryover" of late-eluting peaks from one injection into the next or even next two or three injections. This is usually apparent from the shape of the peak (broad).

The other possibility is "carryover" due to contamination from an excessive amount of drug in the previous sample, either through the syringe or by drug being retained in the injection port or column. This is controlled by the fact that all positive screens are repeated with a new aliquot, to quantitate. Also, the original screen results are compared with the quant results. Any discrepancy is resolved, usually by repeating the extraction. Any samples with large concentration of drug are rescheduled with dilutions, to reduce the amount to within the linear range of the method. If it appears that carryover of this type has occurred, the reviewer will reschedule the case after the overload to verify that there was no contamination.

Frequent change of the autosampler methanol wash bottles helps to control this type of potential carryover.

## ACCEPTANCE CRITERIA

1. External or internal controls must be analyzed in accordance with the standard laboratory procedures to determine the target and acceptable range before being incorporated into routine analysis of the specimens.
2. None of the target analytes should be detected in the negative control (blank).
3. Retention times of target analytes should match those of the calibrator or controls within  $\pm 2\%$ .
4. The positive controls, unless otherwise specified, should be within  $\pm 20\%$ , for blood batches, or  $\pm 30\%$ , for tissue batches, of the established target for all analytes detected in the samples. In other words, if amitriptyline, nortriptyline and methadone are present in the samples, the respective components of the controls must meet the acceptance criteria, but it is not necessary for the other compounds in that same control to meet the acceptance criteria. If the QC fails for quantitation, but is correct qualitatively, a batch can be accepted for qualitative results only. Acceptance of controls outside of  $\pm 20\%$  blood or  $\pm 30\%$  for tissues, of the established target require authorization of the supervisor and documentation.

## DATA REVIEW

### (Second level review)

1. Check chromatography of all injections. Examine the peak shape and note if any peaks show non-Gaussian shape. Identify unresolved peaks and peaks with shoulders on either side. Address any noted problems before proceeding with data review. Consult a supervisor about any unusual events.
2. Check that all significant peaks in the chromatogram are integrated. If the printout allows, check if the baseline used to integrate is appropriate. The analyst should consult with a supervisor about any unusual events, such as the presence of overload peaks
3. Check that all components of each calibrator are present and that each peak is properly assigned. Correct any errors. If any calibration compounds do not extract, or if unexpected multiple peaks are detected for a calibration compound, the analyst must consult with a supervisor.
4. Check the blank for significant peaks that might co-elute with a compound of interest. There is some room for judgment in this step, but the analyst should consult with a supervisor if there are ANY questions. A blank may be scheduled for mass spectrometry to discover the identity of peaks in a blank, but this step must have no bearing on the decision whether a blank is acceptable or not. If any significant peaks are present in a blank for the batch, the run must be rejected.
5. Review QC samples, determine if the controls meet all acceptance criteria and verify the concentration of the components.
6. A copy of the batch calibrators and controls are forwarded to the QC officer, who will enter the results of the QC sample in the QC database. If the batch fails, annotate sample chromatograms, listing the reason for failure.



7. Annotate the following information on the first calibrator's chromatogram or sequence list:
  - a. Calibrators and blank reviewed and accepted (or not accepted). Any QC or calibrator failures are annotated on the proper chromatograms.
  - b. If the files did not transfer, do so, and notify supervisor.
  - c. Note any deviations from the SOP or any comments in a concise but detailed fashion.
  - d. Initial and date.
  - e. Regression coefficient ( $r^2$ ) must be greater than or equal to 0.98.
8. Make sure that each control has the appropriate target concentration range label.

**Note:** *By initialing and dating this document, the analyst certifies that a complete and accurate review was done, to the best of the ability of the analyst.*

9. A supervisor or qualified staff reviews calibrators and controls, dates and initials the results of the review before photocopying.
10. When possible, all data should be photocopied using double sided mode. A master copy of the sequence list, calibration, control and any other data is prepared. Arrange this master copy as follows (from top to bottom): Calibrators, all QC samples and blanks,  $r^2$  report, sequence list and the lot sheet form. It is the responsibility of the analyst to verify that the master copy is legible and is an accurate copy of the originals, with no information cut off at the margins. Photocopies are made from this master, one for each case in the batch. Attach a copy of the calibrator and control package to each original case chromatograph.

## **DATA REVIEW**

### **(Third level review)**

#### **Quantitative Results**

1. The reviewer should review the calibration, control, and batch information received after the photocopying of the batch. Use the same criteria as listed in batch review, with the additional criteria that the  $r^2$  of the four-point calibration curve of each component present in any case in the batch must be equal to or greater than 0.98. Notify a supervisor about any errors and make sure they are corrected before proceeding.
2. Retrieve the case file for each sample in the batch and associate it with the chromatogram(s).
3. Review the case's chromatogram. Determine if the results are consistent with all the other applicable case data to date. For quantitation, it is important to compare the first chromatogram (or the SCAN) results with the final quantitative results. The ratio of peak to internal standard for both chromatograms should be examined. If the results are consistent, report the results on the Result Summary Sheet. Date and initial both the

chromatogram and the Result Summary Sheet. If the results are not consistent, the inconsistency must be resolved. Consult a supervisor about rescheduling the sample, or other appropriate corrective action.

4. If the chromatogram shows overloaded peaks, or poor recovery of the internal standard, then repeating the sample may be necessary. If the problem is an overload, re-schedule with appropriate dilutions. In cases of poor recovery, the sample, its control, blank, and a calibrator may be re-injected once. If the criteria as listed in batch review are still not met, the sample is re-scheduled. If the criteria as listed in batch review are still not met, the sample is re-extracted. If any cases have an unknown or unidentified peak not observed in previous scan results, the case vials must be transferred to GC/MS for identification. All chromatograms must be attached to the case file. Consult with the supervisor if there are any questions. All chromatograms must be attached to the case file. Consult with the supervisor if there is any question.

**Note:** Do not discard any sample paperwork.

5. Cases where an overdose is suspected are scheduled for further testing. Liver and gastric contents are the specimens of choice. Consult with the supervisor regarding tissue analysis.

## REPORTING

1. All control acceptance criteria must be met.
2. Only specimens that have been run along with acceptable controls may be reported. Report concentrations equal to or higher than 50% of the lowest acceptable control or the lowest acceptable calibrator. Concentrations equal to or greater than 25% of the lowest control or lowest calibrator but lower than 50% of the lowest acceptable control or the lowest acceptable calibrator are reported as "less than X" where "X" equals the concentration of the lowest acceptable calibrator or control. Concentrations lower than 25% of the lowest acceptable control or calibrator are reported as "not detected".
3. All positive findings are reported to two decimal points (e.g., "lamotrigine 0.82 mg/L").
4. Drugs detected at a concentration above the upper limit of quantitation must be reported as "greater than...", unless the concentration was determined by repeat analysis using appropriate dilutions. Diluting until the sample is within the linear range of the analysis is the most desirable result, but if the sample is insufficient, or other considerations preclude dilution, report as above.

Drug facilitated sexual assault cases and driving under the influence cases have different reporting criteria. Results are reported in ng/mL.

## REFERENCES

SPEware Corp. Cerex Applications Manual.

System 48 Processor. Users Guide.

Turbovap. Users Guide.

Uncontrolled Copy